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Infrared spectroscopy and multivariate analysis to appraise α -cellulose extracted from wood for stable carbon isotope measurements

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Some practical details for the cellulose extraction method

Before beginning the chemical extractions, the powdered wood samples are oven dried at 60°C for 24 h. Then, 0.15 g of the sample material is put into individual pouches made of PTFE pre-cut filter membranes with a pore size of 1 μm (Alltech, France). The filter membrane is shaped into a pouch which are closed with a Teflon ribbon and identified with coloured glass beads on the ribbon ("sample pouches"). For several steps of the α -cellulose extraction process (Fig.1 in the published article), samples are put in an ultrasound water bath at 45 Hz (VWR cleaner) ("ultrasound bath"). The quantities in the following method description were used for sample pouches containing each 0.15g of dry wood material and a number of pouches according to the glassware used in each step. Quantities of solvents and products need to be adjusted to other sample quantities.

(1) Extractives free. One hundred and twenty sample pouches are placed in three Soxhlet extractors (around 40 sample pouches, thus together 6g of wood material in a 125 mL extractor bulb) with each a 2:1 (v/v) 95% toluene: 95% ethanol mixture (for 5 successive cycles) and then with 99.8% ethanol (for 3 successive cycles). Sample pouches are then removed and placed in 99.8% ethanol and subjected to the "ultrasound bath" at room temperature for 10 minutes. Then, the sample pouches are removed from the ethanol bath, put on a flat surface under a fume hood for one hour to remove the solvents. Next, the same sample pouches are boiled in distilled water into two beaker each containing 1L of distilled water (~60 pouches in each beaker). Each beaker is covered with watch glass and placed into a water bath for 3 hours followed by the "ultrasound bath" at room temperature for 10 minutes in warm distilled water.

(2) NaClO₂ delignification. The sample pouches are then transferred into two Erlenmeyer flask each containing 800 mL of distilled water (~60 pouches representing 9g of original wood material), where the pH value

is adjusted between 3 and 4 by adding glacial acetic acid. Then, the lignin digestion is started by adding 21 g of NaClO₂ ($\approx 2.45\text{g/g}$ of wood) to each flask. These flasks are placed on a hot plate at 70°C, and solutions are shaken under magnetic stirrer for 14 hours, to enable a complete penetration of the liquid into the sample pouches. The flasks are covered to allow vapour condensing, but not tightly, to avoid a pressure increase. Then the flasks containing the sample pouches are moved to the "ultrasound bath" at 70°C till the end of the experiment. Subsequently, depending on the chemical treatment performed (Table 1 in the published article and see published article for data for oak, beech, pine and poplar), one and up to four more additions of 21 g of NaClO₂ are made to each Erlenmeyer flasks, with a time of two hours between every addition. Before each addition of NaClO₂, the pH value is adjusted to values between 3 and 4 by adding glacial acetic acid. Two hours after the last addition of NaClO₂, the sample pouches are removed and washed once with 1L of distilled water in a beaker subjected to the "ultrasound bath" at room temperature for 10 minutes, then washed for 14 hours with 2 L of distilled water in a beaker using a magnetic stirrer and again during one hour under continuous fresh distilled water flow in a 2 L-side-arm flask. If this state of cellulose extraction (usually called holocellulose) is sufficient, the sample pouches are removed from the distilled water, dried with 99.8% ethanol in a beaker in the "ultrasound bath" at room temperature for 10 minutes. Sample pouches are removed from ethanol and put on a flat surface for mechanically dried under a fume hood for several hours, then opened, and the extracted cellulose is transferred to Eppendorf sampling tubes, in which the samples are dried (at least 36 h for complete dryness) in an oven at 60°C.

(3) Alkaline NaOH hydrolysis. To achieve analytical α -cellulose (subtract some residual hemicelluloses) an additional step of alkaline hydrolysis is sometimes necessary (see published article for data for oak, beech, pine and poplar). To avoid the formation of carbonates from atmospheric CO₂ dissolved in the sodium hydroxide solution, all NaOH solution preparation steps are placed under "ultrasonic bath". Step one: a beaker with 1L of distilled water is already subjected to the "ultrasonic bath" for 5 min, prior to adding the soda. Step two: always under "ultrasonic bath" appropriate sodium hydroxide pellet according to required 10% of sodium hydroxide solution is added in the beaker. Step three: 5 min after the soda addition, the sample pouches are introduced in sodium hydroxide solution to react under "ultrasonic bath" for 1 hour (each beaker containing ~ 60 pouches representing 9g of original wood material). The sample pouches are then removed from the beaker and washed once with 1L of distilled water in a beaker subjected to the "ultrasound bath" for 10 min. Next, the sample pouches are placed in 1L of distilled water and the pH is adjusted to 7-8 using 10% hydrochloric acid solutions under "ultrasound bath" for 30 min.

The sample pouches are then removed and washed thrice with 1L of distilled water in a beaker subjected to the "ultrasound bath" for 10 min, They are dried with 99.8% ethanol in a beaker subjected to the "ultrasound bath" for 10 min and then under a hood with ventilated air to several hours. Then, the samples pouches are opened and the extracted cellulose is removed from the pouches and oven-dried in 1ml Eppendorf tubes at 60°C for 36 h.